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Antiviral prophylaxis with twice daily topical cidofovir protects against challenge in the adenovirus type 5/New Zealand rabbit ocular model

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Abstract

Adenoviral ocular infections are the most common external ocular infections world wide and there is no approved treatment. Topical cidofovir has been shown to be effective in vitro, in animal models and in case studies for the treatment of adenoviral ocular infections. Prophylaxis to prevent transmission within households and to reduce community epidemics remains an important public health goal. The current study examined whether antiviral prophylaxis with cidofovir, twice daily dosing, would restrict viral replication following a large challenge inoculum of adenovirus type 5 (Ad5) in the New Zealand white rabbit ocular model. The results showed that antiviral prophylaxis with 1 and 0.5% cidofovir significantly reduced mean daily Ad5 ocular titers (days 0–5), the number of Ad5 positive cultures/total (days 1–14), serial Ad5 positive cultures/total (days 1, 2, 3, 4, 5, 7), and the number of eyes with Ad5 replication beyond day 0 (1% cidofovir only). Antiviral prophylaxis appears to be an effective strategy to reduce and restrict adenovirus replication experimentally.

Keywords: Antiviral drugs; Adenovirus; Cidofovir; Epidemic keratoconjunctivitis

1. Introduction

Adenoviruses (Ad) are the etiologic agents that cause the most commonly occurring external ocular viral infection worldwide. Ocular adenovirus infections are associated with significant patient morbidity, including symptomatic distress with visual disturbances that can last months to years. Three forms of adenovirus ocular infections are: epidemic keratoconjunctivitis (EKC), pharyngeal conjunctival fever, and follicular conjunctivitis. These highly contagious infections frequently occur in epidemics at places where people are in close contact, such as home, schools, military bases, and ophthalmic medical facilities (Gordon et al., 1996a). At present, there is no FDA-approved antiviral treatment for adenovirus ocular infections.

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Cidofovir [(*S*)-HPMPC] is a broad spectrum antiviral with significant in vitro inhibitory activity against a number of DNA viruses (HCMV, HSV-1, HSV-2, VZV, and adenoviruses) (De Clercq, 1993). Prior studies using topical cidofovir in various treatment regimens significantly reduced Ad5 (Gordon et al., 1994; de Oliveira et al., 1996; Romanowski and Gordon, 2000; Gordon et al., 1992), Ad2 (Romanowski and Gordon, 2000), and Ad6 (Romanowski and Gordon, 2000) ocular titers and the duration of adenovirus shedding in the New Zealand white (NZW) rabbit ocular model. Topical cidofovir has been shown to be both safe and effective for treating adenovirus ocular infections in case studies (Gordon et al., 1996b; Gordon, 1998).

As cidofovir in its depot form (cidofovirphosphate-choline) persists in treated cells for extended periods of time (intracellular half-life 24–65 h) (Naesens et al., 1997), the establishment of therapeutic levels of cidofovir using infrequent dosing at higher concentrations seems a desirable clinical strategy. Infrequent dosing is associated with improved patient convenience and better compliance. The goal of the current study was to determine whether antiviral prophylaxis with topical cidofovir using infrequent dosing (twice daily) would protect against adenovirus challenge in the Ad5/NZW rabbit ocular model.

2. Materials and methods

2.1. Virus and cells

A clinical adenovirus isolate was cultured from a patient presenting with typical adenovirus keratoconjunctivitis at the Eye & Ear Institute of Pittsburgh. The isolate was serotyped by serum neutralization, and found to be type 5. The Ad5 isolate was grown in A549 monolayers, harvested, aliquoted, and frozen as stock virus at -70 °C. Prior to use, the stock viruses were titered using a standard plaque assay.

A549 human lung carcinoma cells, (CCL-185, American Type Culture Collection, Manassas, VA), were grown in Eagle's minimum essential medium with Earle's salts, supplemented with 6% fetal bovine serum, 2.5 μg/ml amphotericin B, 100 units/ml penicillin G, 0.1 mg/ml streptomycin, and 0.5 mg/ml gentamycin (Sigma Cell Culture Reagents, St. Louis, MO).

2.2. Experimental drugs

One and 0.5% cidofovir [(*S*)-HPMPC, (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine] solutions were prepared in PBS from the 7.5% injection form of cidofovir (Vistide, Gilead Sciences, Inc., Foster City, CA). PBS served as control drops.

2.3. Animals

Two- to three-pound female New Zealand albino rabbits were purchased from Myrtle's Rabbitry, Thompson Station, TN. All animal studies conformed to the ARVO statement on the Use of Animals in Ophthalmic and Vision Research. University of Pittsburgh Institutional Animal Care and Use Committee (IACUC) approval for this study was obtained and institutional guidelines regarding animal experimentation were followed.

2.4. Experimental design

This study was performed in duplicate using 21 rabbits per experiment. As eye to eye transmission of adenovirus does not occur in the Ad5/NZW rabbit ocular model, a pretreatment-challenge model was used to evaluate antiviral prophylaxis. Two days prior to virus inoculation, rabbits were randomly assigned to one of three topical ocular therapy groups ($n = 14$/group) and twice daily bilateral treatment initiated: (I) 1% cidofovir, (II) 0.5% cidofovir; (III) PBS (Control). On the day of challenge inoculation, 2 h following the first instillation of the experimental drugs, anesthetized rabbits were inoculated with 50 μl of Ad5 (1.2×10^5 pfu/eye) in both eyes after 12 cross-hatched strokes of a no. 25 sterile needle. The second instillation of the experimental drugs was performed 2 h post-inoculation. Twice daily therapy was continued for 4 additional days for a total of 7 days of topical treatment. Ocular swabbing to

recover adenovirus from the tear film, and corneal and conjunctival surfaces was performed on days 0, 1, 2, 3, 4, 5, 7, 9, 11, and 14 after inoculation at least 1 h following the final instillation of test drugs. The samples were frozen at −70 °C pending plaque assay.

2.5. Determination of ocular viral titers (plaque assay)

The ocular cultures to be titered were thawed, diluted (1:10), and inoculated onto A549 monolayers. The virus was adsorbed for 3 h. Following adsorption, 1 ml of media plus 0.5% methylcellulose was added to each well, and the plates were incubated at 37 °C in a 5% CO_2–water vapor atmosphere. After 7 days incubation, the cells were stained with 0.5% gentian violet, and the number of plaques were counted under a dissecting microscope (25 ×). The viral titers were then calculated, and expressed as plaque-forming unit per milliliter (pfu/ml).

2.6. Statistical analysis

Following the completion of both trials, the codes were broken, and the data from each experiment were analyzed statistically using Minitab (Minitab Inc., State College, PA) and True Epistat (Epistat Services, Mesquite, TX) statistical software. As comparable results were obtained in each experiment, the data were then pooled to obtain a larger subject number, and analyzed using analysis of variance (ANOVA) using Tukey's pairwise comparisons, Monte Carlo Randomization, and χ^2 analyses. Significance was established at the $P \leqslant 0.05$ confidence level.

3. Results

3.1. Ad5-positive cultures per total

The number of Ad5-positive cultures per total was determined for the treatment groups by ascertaining the number of eye swabs that demonstrated a positive culture per total number of cultures. Table 1 summarizes these results. Overall from days 1–14, eyes treated with 1% cidofovir demonstrated fewer Ad5-positive cultures (29/252 [12%]) compared to the Control group (177/252 [70%]; $P < 0.05$) and the 0.5% cidofovir group (81/252 [32%]; $P < 0.05$). In addition, the 0.5% cidofovir group also demonstrated fewer Ad5-positive cultures compared to the Control group ($P < 0.05$) (χ^2 analysis).

Daily Ad5-positive cultures are also presented in Table 1. Treatment with 1% cidofovir significantly reduced the number of Ad5-positive cultures compared to the Control group on days 1, 2, 3, 4, 5, 7, 9, and 11 ($P \leqslant 0.007$). Treatment with 0.5% cidofovir significantly reduced the number of Ad5-positive cultures compared to the Control group on days 1–5, and 7 ($P < 0.03$). In addition, the 1% cidofovir group also demonstrated fewer Ad5-positive cultures compared to the 0.5% Cidofovir group on days 2, 3, 5, 7, 9, and 11 ($P < 0.05$) (χ^2 analysis).

3.2. Adenovirus ocular titers

The mean serial Ad5 ocular titers for all treatment groups were determined by calculating the mean and standard deviation of all ocular cultures ($n = 28$ per group) per day. Compared to the Control group, treatment with 1% cidofovir significantly reduced Ad5 titers on days 0, 1, 2, 3, 4,

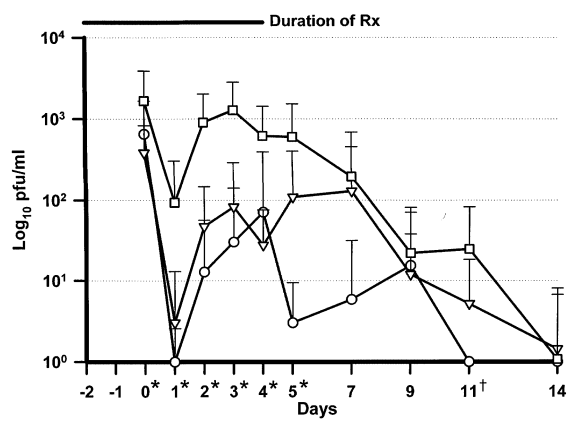

Fig. 1. Demonstrates the mean and standard deviation of Ad5 ocular titers for each culture day. The (*) denotes the days on which both the 1% (○) and 0.5% (▽) cidofovir treatment groups demonstrated significantly lower mean Ad5 titers compared to the Control (□) group.

Table 1
Adenovirus-positive cultures per total, days 1–14

Treatment	Day									Total
	1	2	3	4	5	7	9	11	14	
1% Cidofovir	3/28* (11)	5/28*,† (18)	4/28*,† (14)	5/28* (18)	6/28*,† (21)	3/28*,† (11)	3/28*,† (11)	0/28*,† (0)	0/28 (0)	29/252*,† (12)
0.5% Cidofovir	4/28* (14)	15/28* (54)	11/28* (39)	11/28* (39)	13/28* (46)	9/28* (32)	10/28 (36)	6/28 (21)	2/28 (7)	81/252* (32)
Control	19/28 (68)	28/28 (100)	28/28 (100)	27/28 (96)	27/28 (96)	23/28 (82)	12/28 (43)	12/28 (43)	1/28 (4)	177/252 (70)

* $P<0.05$ when compared to Control group (χ^2 analysis).
† $P<0.05$ when comparing the 1% Cidofovir group to the 0.5% Cidofovir group (χ^2 analysis).
Percentages are in parentheses.

5, and 11 ($P \leqslant 0.022$) (ANOVA). Similarly, treatment with 0.5% cidofovir significantly reduced Ad5 titers on days 0, 1, 2, 3, 4, and 5, ($P \leqslant 0.007$) compared to the Control group. There was no difference between the cidofovir treatment groups on any of the culture days. These results are graphically displayed in Fig. 1.

3.3. Prevention of replication

The ultimate measure of ocular antiviral prophylaxis is whether a potential infection can be prevented in eyes that received pretreatment. We determined the number of eyes per total in which replication was prevented for each group by calculating the number of eyes that did not demonstrate any positive Ad5 cultures beyond the day 0 culture in which only residual inoculum virus was recovered. Replication was completely prevented in 13 of 28 eyes (46%) treated with 1% Cidofovir. This was significantly greater than the 4 of 28 eyes (14%) in the 0.5% Cidofovir group and the 0 of 28 eyes (0%) in the Control group ($P \leqslant 0.009$) (χ^2 analysis). There was no difference between the 0.5% Cidofovir group and the Control group (Monte Carlo Randomization Test).

3.4. Ocular toxicity

No significant local ocular toxicity was observed for 1 and 0.5% cidofovir during the course of the study.

4. Discussion

The highly contagious nature of adenovirus ocular infections put individuals in close contact with infected patients at high risk to contract the disease. Effective prophylaxis would not only protect at risk family members, health care workers, teachers, and fellow workers during epidemics, but should also reduce patient morbidity by preventing transmission to the fellow eye.

Immunization with live oral vaccines aimed at Ad4 and Ad7 has effectively reduced the incidence of febrile respiratory infections in the military (Barraza et al., 1999). However, there are no vaccines directed against the major ocular serotypes of adenovirus (Ad8, Ad19 and Ad37).

Antiviral prophylaxis represents an alternative strategy to prevent or lessen the severity of infections in high risk individuals. Acyclovir (Mertz et al., 1988), valacyclovir (Baker et al., 1999) and famciclovir (Daiz-Mitoma et al., 1998) have been used successfully applied to suppress recurrences of genital herpes infections and so has acyclovir for ocular herpes infections (Herpetic Eye Disease Study Group, 1998). At present there is no approved systemic or topical antiviral agent available for the prophylaxis or treatment of adenovirus ocular infections.

Cidofovir, a broad spectrum topical antiviral, has demonstrated a significant reduction of adenovirus replication (Ad2, Ad5, Ad6) in previous studies in the NZW rabbit ocular model (Gordon et al., 1994; de Oliveira et al., 1996; Romanowski and Gordon, 2000; Gordon et al., 1992) and clinical efficacy in patients (Gordon et al., 1996b; Gordon, 1998). In the current study, antiviral prophylaxis with infrequent (twice daily) topical dosing of both 0.5 and 1.0% cidofovir reduced adenovirus replication following a significant adenovirus challenge in the Ad5/NZW rabbit ocular model. This study was designed to test cidofovir prophylaxis using a large Ad5 challenge inoculum (1.2×10^5 pfu/eye). In the clinical setting however, the titer of the challenge inoculum will be unknown. Adenovirus clinical isolates routinely recovered by ocular swabbing in the Charles T. Campbell Ophthalmic Microbiology Laboratory have titers in the range of 10^2–10^4 pfu/eye. Presumably, the titer of the infecting inoculum in at risk individuals will be less than the large challenge inoculum used in the current study. Therefore, ocular infection may be effectively prevented by the lower concentration of 0.5% cidofovir.

The overall safety of 0.5% cidofovir administered topically twice daily for 7 days was established in Phase I and II clinical trials, (Gordon, 1998). However, recent off-label usage of higher doses of topical cidofovir for more than 1 week (Hawaii, Germany) has been associated with lacrimal canalicular blockade, a complication previously reported in the Ad5/NZ rabbit ocular

model (Gordon et al., 1994). Concerns about the narrow window of efficacy versus toxicity for cidofovir has led Bausch & Lomb Pharmaceuticals to abandon its development program and return the drug to Gilead Sciences Inc., Foster City, CA, where its future for topical ophthalmic use remains uncertain.

Nevertheless, the results of the current experimental study have established 'a proof of principle' that antiviral prophylaxis for ocular adenovirus infection appears to be an achievable goal. Clinically, antiviral prophylaxis may prove to be an effective strategy to reduce adenovirus transmission to the fellow eye and to at risk susceptibles.

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